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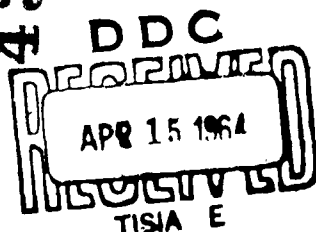
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THE PHYSIOLOGY OF CRUSTACEAN INTERNEURONS

Donald Kennedy

Department of Biological Sciences

Stanford University, Stanford, California



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INTRODUCTION

The two-year grant reported here was designed to equip and support a visiting post-doctoral investigator in this laboratory to work in collaboration with the principal investigator on problems relating to the physiology of central interneurons in crustacea and of the afferent fibers which supply excitation to them. In the original proposal two avenues of investigation were laid out. The first of these involved studies on the photosensitivity of crustacean interneurons, especially that of the pair of nerve fibers in the abdominal nerve cord of the crayfish which had been previously shown to be light-sensitive. This segment of the work was completed somewhat earlier than anticipated, and largely on the basis of previously available support from the U.S. Public Health Service. These experiments quickly reached a point at which further effort was not deemed profitable, and this line of investigation has therefore not been pursued further. Five copies of the publication which resulted from these studies are enclosed--not because the work was accomplished primarily under the tenure of this grant, but because it explains the termination of this particular line of investigation.

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The major proportion of the original proposal involved further studies on the central connections of crustacean interneurons, and the properties of the synapses between afferent fibers and interneurons. These studies have been concentrated on under the tenure of this grant. In addition, each of the visiting investigators brought to Stanford under the grant have pursued distinct but related programs of investigation of their own in addition to the collaborative work on the interneuron problem. This report will consider both phases of activity.

PERSONNEL

Two research associates have been supported in this laboratory under the grant. The first is Dr. DeForest Mellon, Jr., who came in the fall of 1961. He was supported between October 1961 and October 1962; he then stayed an additional year in the laboratory with other support to complete some of the investigations which he had begun. He has since been appointed assistant professor of biology at the University of Virginia, and now is actively pursuing several problems which have grown out of the projects he worked on here. I mention this because it seems to me a significant result of this support that a multiplication of laboratories actively working on the problem has been made possible.

The second research associate, appointed in October 1962, is Dr. Kimihisa Takeda, Instructor in Physiology at the University of Tokyo Medical School. Dr. Takeda has remained here for a second year, supported under a continuation of the same grant.

The Physiology of Crayfish Interneurons

We have concentrated our efforts on two related problems. The first of these concerns the organization of input to, and the central coding of sensory information in, a numerically large class of interneurons which receives synaptic input in several different body segments. This work, pursued intensively with Dr. Mellon during his two years here, has clarified our knowledge of the mechanism by which impulses are initiated by presynaptic events in the branching-fiber system within central neuropile. In addition, we have been able to analyze the properties of impulse collision and refractoriness which result from the fact that impulses are being initiated in several different regions of the same axon. In particular, we have been able to use such neurons as models for a new mechanism whereby the pattern of impulses in a single neuron might indicate information about dynamic stimulus parameters: in particular, velocity and direction. The results of this work were presented at a symposium on neural theory and modeling held at Ojai, California in December of 1962. They will shortly be published in the Proceedings of that Conference by Stanford University Press, and the reference is included on the list of publications. A summary of some of the results will be found in the accompanying abstract of a paper presented at the AAAS meetings in Cleveland last December. A full manuscript describing recovery-cycle properties of the synapses involved and experiments on the mechanism of impulse initiation, as well as the interactions between different spike-generating loci, has been submitted to the Journal of Neurophysiology.

This investigation is continuing under the continuation of this grant in collaboration with Miss Joan Johnston, a terminal graduate student in this laboratory, and with Dr. Takeda. It is expected that the analysis can be made

more complete since we now have techniques for marking the position of intracellular recording electrodes by electrophoretic deposition of dyes from the microelectrode, as well as for passing current through the cell membrane while recording.

The Properties of Bipolar Sensory Neurons

Dr. Mallon began an investigation of some of the natural sensory inputs to the interneurons studied, and in the course of doing so developed a new preparation of considerable interest. He was able to show that tactile hairs on the exoskeleton of crayfish are of several classes, and that in one of these the sensory elements are innervated by two receptor neurons; one of these is sensitive to movement in one direction, and the other to the opposite movement. The general properties of these receptors, and their possible importance to the organism in interpreting its environment, are treated in a paper published in the Journal of Experimental Biology. Five reprints of this publication are enclosed with this report.

The receptor neurons belonging to this system are of sufficient size to make possible, for the first time, decisive experiments on the point of impulse initiation in this type of neuron. Microelectrode recording from the somata of these cells reveals that the spike is initiated at a rather distal locus on the dendrite itself, and thus that these dendrites are capable of supporting all-or-none activity. It was also shown that the presence of the soma as an expansion in the conducting pathway limits the frequency response of these neurons, since the dendrite-soma boundary is a critical site of low safety factor. This circumstance leads naturally to a possible explanation of why, in most "advanced" neurons of this type, the soma is

placed off the main conduction pathway on a side branch (e.g., invertebrate central neurons, vertebrate spinal ganglion cells). This aspect of the work has just been published in the Journal of General Physiology; reference is made to this paper in the list of publications, and reprints of it will be sent as soon as they become available.

Responses of Crayfish Motoneurons

Dr. Takeda's major project, aside from our collaborative work on interneurons, has dealt with the problem of the activation of motor neurons by other central elements. He has developed a technique for penetrating, under visual control, large cell bodies located on the ventral surface of the third abdominal ganglion of the crayfish with two microelectrodes, one for recording membrane potentials and the other for passing current. These cells can be identified as flexor motoneurons by antidromic stimulation of the third root, an exclusively motor nerve which innervates abdominal flexor muscles. The results of these experiments confirm that the soma, which is devoid of synapses, is located at a considerable distance from the site of synaptic impingement on branches of the axon in the neuropile. Electrical activity in the somata of such motoneurons, evoked by stimulation of giant fibers in the nerve cord, consist of three components. The first is a small, electrotonic potential associated with activity in several branches of the main axon. The second is associated with the efferent spike in the third root, and is the response of the main axon. The third component represents invasion of the impulse into the soma. Third component is easy to block by slight hyperpolarization of the soma; when this is accomplished there is no effect on the efferent spike, which demonstrates that the soma is uninvolved in the transmission pathway. When the second component is blocked by deeper hyperpolarization, the efferent spike is blocked also. The first component cannot be

blocked even by ^{very deep} ~~varying~~ hyperpolarization; but it does not show a significant increase in amplitude, as would be expected if it were a "pure" synaptic potential. A number of flexor motor neurons are excited by a single impulse in any one of the four central giant fibers. In addition, excitatory and inhibitory pathways exist both in the connectives of the ventral nerve cord and in the segmental first and second roots to the ganglion in which the soma lies. Thus these neurons perform a unique integrative task: they receive highly effective, individual presynaptic inputs from the giant fibers, in response to which they act as simple relays, and in addition respond to a highly convergent set of small-fibered afferent pathways. An important part of the mechanism of integration in these cells is the presence of all-or-none responses in one or several individual branches of the main axon, which may have to summate to produce discharge. Thus, in addition to the conventional addition of graded synaptic potentials, a second stage of integration exists between impulse events in branches and discharge of the main axon. Dr. Takeda has also analyzed the electrical properties of the cell bodies. They prove to be electrically excitable, a fact which was not previously known, and their membrane constants have been calculated.

A limited number of motoneurons operate the flexor muscles of each segment. Of the ~~gen~~ which pass out each third root, these experiments have individually identified at least six. Identification of these motoneurons and localization of their somata has made possible a new set of experiments which are in progress now. The cells can be individually stimulated, either in the third root or by intercellular stimulation of their soma, and this is enabling us to work out the actions of each on the segmental musculature. As is well known, several types of motoneurons are found in arthropod (and

other invertebrate) systems. Some produce "fast" junctional potentials, often with secondary responses, which produce twitch-like contractions in the muscle. Others produce "slow" junctional potentials which require facilitation in order to achieve tension development. Still others are inhibitory. We are currently trying to take advantage of this new understanding of the inputs to individually-specified motoneurons in order to untangle the details of the neural actions which underlie specific reflex behavior.

Five copies of an abstract resulting from the motoneuron experiments are enclosed. A full manuscript has been prepared and submitted to the Journal of Neurophysiology, and reprints will be sent when they become available.

Publications Resulting from This Research

1. Mellon, DeF. Jr. and Kennedy, D. 1962. Response patterns from the thoracic tactile receptors of the crayfish. *Amer. Zool.* 2:431.*
2. Mellon, DeF. Jr. 1963. Electrical responses from dually-innervated tactile receptors of the thorax of the crayfish. *J. Exp. Biol.* 40: 137-148.
3. Mellon, DeF. Jr. 1963. Impulse initiation in bipolar sensory neurons. *Fed. Proc.* 22:174.*
4. Mellon, DeF. Jr. and Kennedy, D. 1964. Impulse origin and propagation in a bipolar sensory neuron. *J. Gen. Physiol.* 47:487-499.
5. Kennedy, D. and Mellon, DeF. Jr. 1963. The organization of input to complex crayfish interneurons. *Amer. Zool.* 3:159.*
6. Kennedy, D. and Mellon, DeF. Jr. 1964. Receptive-field organization and response patterns in neurons with spatially-distributed input. In: Neural Theory and Modeling, R. F. Reiss, ed. Stanford University Press, 1964. In press.
7. Takeda, K. and Kennedy, D. 1963. The property of crayfish motoneurons. *Amer. Zool.* 3:158.*
8. Takeda, K. and Kennedy, D. 1964. Some potentials and modes of activation of crayfish motoneurons. Submitted to *Journal of Neurophysiology*.
9. Kennedy, D. and Mellon, DeF. Jr. 1964. Synaptic activation and receptive fields in crayfish interneurons. Submitted to *Journal of Neurophysiology*.

* Abstract